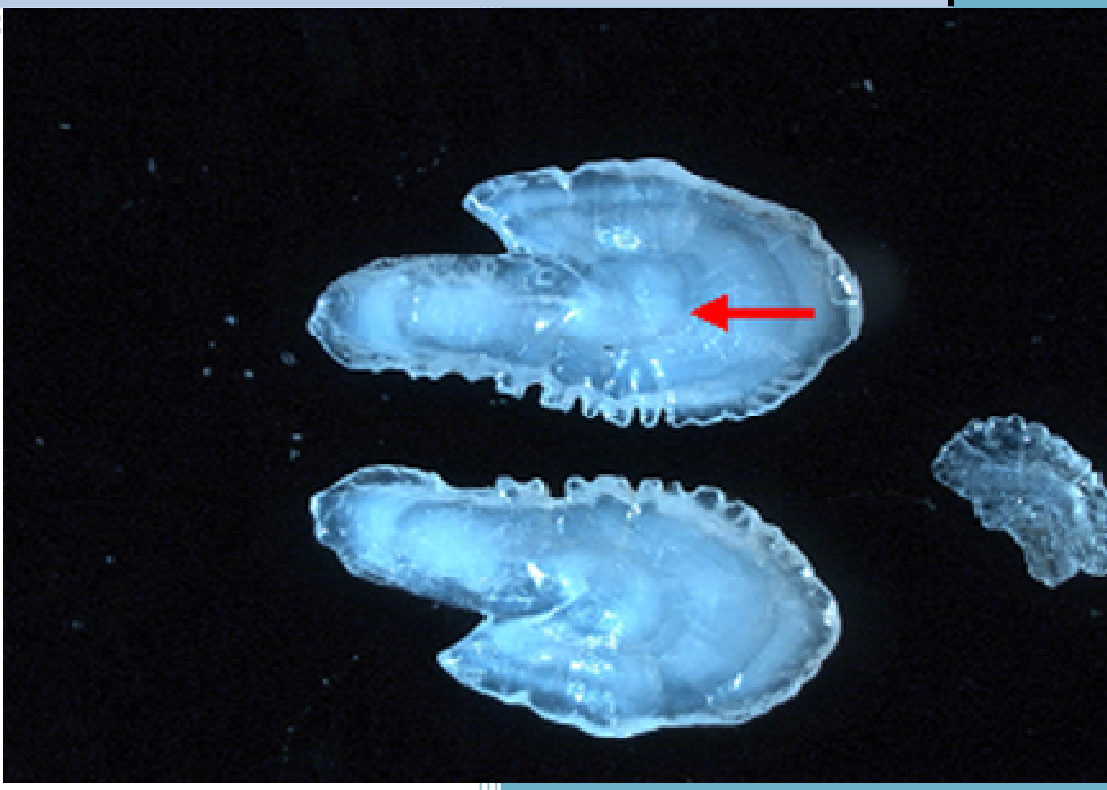
02

Report of the AdriaMed Workshop on Inter-calibration of Sardine Otolith Reading in the Adriatic Sea (Split, Croatia, 28-29 June 2011)



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GCP/RER/010/ITA/SR-02

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Preface

The Regional Project “Scientific Cooperation to Support Responsible Fisheries in the Adriatic Sea” (AdriaMed) is executed by the Food and Agriculture Organization of the United Nations (FAO) and funded by the Italian Ministry of Agriculture, Food and Forestry Policies (MiPAAF) and since 2007 from the Directorate General for Fisheries and Maritime Affairs of the European Commission.

AdriaMed was conceived to contribute to the promotion of cooperative fishery management between the participating countries (Republics of Albania, Croatia, Italy, Montenegro and Slovenia), in line with the Code of Conduct for Responsible Fisheries adopted by the UN-FAO.

Particular attention is given to encouraging and sustaining a smooth process of international collaboration between the Adriatic Sea coastal countries in fishery management, planning and implementation. Consideration is also given to strengthening technical coordination between the national fishery research institutes and administrations, the fishery organizations and the other relevant stakeholders of the Adriatic countries.

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Occasionally, relevant documents may be translated into national languages as AdriaMed Translations (GCP/RER/010/ITA/AT-00).

Comments on this document would be welcomed and should be sent to the Project headquarters

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Preparation of this document

This document is the final version of the Report of the AdriaMed Workshop on inter-calibration of sardine otolith reading, organized by the FAO AdriaMed Project (***Scientific Cooperation to Support Responsible Fisheries in the Adriatic Sea)*** and held in Split, Croatia from 28 to 29 June 2011.
The reports summarizes the main results of the workshop which aimed at providing at addressing some problems related to the age determination of sardine by otolith reading.
In the framework of the AdriaMed research programme on small pelagic fisheries resources, the report is intended to contribute to establishing a standardised procedure for the age determination of sardine through otolith reading in the Adriatic Sea coastal countries (Albania, Croatia, Italy Montenegro and Slovenia).

Acknowledgements

The Institute of Oceanography and Fisheries of Split, which through Ms Ivona Marasović, provided kind hospitality and assistance in the organization of the Meeting, is gratefully acknowledged.

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ABSTRACT

The AdriaMed Workshop on inter-calibration of sardine otolith reading in the Adriatic Sea was organized in Split, Croatia from 28 to 29 June 2011. The Workshop was funded and organized by the project GCP/RER/010/ITA FAO AdriaMed “Scientific Cooperation to Support Responsible Fisheries in the Adriatic Sea” kindly hosted by the Institute of Oceanography and Fisheries of Split. Altogether twelve (12) scientists from five Adriatic countries and FAO staff participated. The Workshop listed and discussed on the main difficulties related to the sardine (*Sardina pilchardus*) otolith microstructure interpretation in the Adriatic Sea and identified a common protocol for readings between the research groups participating in the AdriaMed Project. The final outcome of the meeting consisted of revised agreed guidelines for the preparation and interpretation of sardine otoliths.

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Report of the AdriaMed Workshop on inter-calibration of sardine otolith reading

Split, Croatia 28-29 June 2011

1. Introduction

Fish resource assessment requires, in some cases, current knowledge on age structure of fish populations in order to provide appropriate advice in support of fisheries management. This requirement implies an accurate determination of fish age for target species and, consequently, the setting-up of suitable methodologies and criteria to be used in common by experts engaged in age reading. The age determination of fish species is a fundamental tool for studying biological aspects such as growth, time necessary to first maturity and life span.
The AdriaMed Workshop on inter-calibration of sardine otolith reading (henceforth referred to as WS) was held at Institute of Oceanography and Fisheries in Split (Croatia) on 28 and 29 June 2011. The workshop was attended by researchers from the Adriatic fisheries research institutes involved in Project activities from Albania, Croatia, Italy, Montenegro and Slovenia as well as staff of FAO AdriaMed Project. The list of all participants is given in Annex 1.
The aim of the workshop was to identify the main difficulties related to the interpretation of sardine otolith macrostructure (annual rings) in the Adriatic Sea and to establish a common protocol for otolith readings for the research groups participating in the AdriaMed Project. With this aim, the WS reviewed the sardine age reading criteria and proposed measures in order to improve the precision among otolith age readings and/or readers in the Adriatic Sea, thus improving the quality of the biological data collected from wild stocks and used in sardine stock assessment.

1.1 Background

Similarly to what happens for a significant number of fish species, age determination of Adriatic Sea sardine is primarily based on the analysis of otolith structure (otolith reading). The standardization of methodology for sardine otolith reading and the improvement of the readings precision in the Adriatic Sea initiated in the 80's. Taking the initiative from such work, the AdriaMed Project, in the framework of the component on small pelagics, strongly supported the standardization of sampling and data collecting methodologies including, among others, the improvement of otolith reading techniques. In recent years this support resulted, for the Adriatic region, in otolith exchanges and in the organization of working groups and workshops focused, ***inter alia***, on sardine and anchovy age determination through otolith reading.
The First Meeting of the AdriaMed Working Group on Small Pelagics Resources held in Split (12-13 October 2000) suggested the biological sampling of shared small pelagic Fisheries resources as one of the specific activities to be implemented within AdriaMed framework (Mannini, ***et al.***, 2001). As follow up a specific research programme was presented to the Project and discussed during the second meeting of the AdriaMed Coordination Committee (Portorož, Slovenia, 22-23 November 2000). The Coordination

Committee included the above-mentioned research programme in the list of the Project activities (AdriaMed, 2001). The AdriaMed Working Groups on Shared Demersal and Small Pelagic Fishery Resources of the Adriatic Sea, held in Bari (13-15 February 2001), suggested implementation of the above-mentioned research programme with the title "Data Collection and Biological Sampling System on Small Pelagics in the Adriatic Sea", (henceforth AdriaMed-SP). Due to the complexity of the programme, it was suggested to limit the target species to anchovy (*Engraulis encrasicolus*) and sardine (*Sardina pilchardus*).

One of the objectives of AdriaMed-SP was the development of a regional system for the monitoring and assessment of the small pelagic fish biomass and related fishery exploitation pattern in the Adriatic Sea. The AdriaMed-SP also aimed to supply the AdriaMed participating countries with dynamic tools for data collection and biological sampling for anchovy and sardine. Other objectives of the AdriaMed-SP were: the establishment of a common sampling protocol in the participating countries; the strengthening of international cooperation between the Adriatic countries; and supporting the national capacity development in terms of expertise through the organization of on-the-job training courses.

The common methodology applied to achieve some of these objectives included support for the collection of commercial catch and effort data by species and gear at selected landing site (base port) around the Adriatic region with associated collection of biological sampling (known as AdriaMed Protocol, Cingolani and Santojanni, 2003). The AdriaMed-SP research programme was operative from May 2001 to May 2005; pursuing this work, AdriaMed coordinated several activities which involved national experts working in several fisheries research institutions from Albania, Croatia, Italy and Slovenia[1]. Several training courses were organized[2] and two Workshops for the joint stock assessment of anchovy and sardines in the Adriatic Sea were held (Italy, May 2010 and May 2011). The research programme produced four regional assessments of small pelagics in GSA 17. Annual estimates of mid-year stock biomass and of mean fishing mortality rates were obtained. The mean ratio between fishery catch and estimated mid-year biomass was assessed for both anchovy and sardine stocks. All of the joint stock assessments were presented and discussed at the GFCM SAC Sub-committee on Stock Assessment meetings (e.g. Spain, November-December 2009; Italy, November 2010; Greece, October 2011). The research programme developed by AdriaMed resulted in the production of five documents published as AdriaMed Occasional Papers.

From 2005 onward, the activities on small pelagic has continued. Problems and issues related to the small pelagic fisheries have been discussed during the annual meetings of the AdriaMed Working Group of Small Pelagic Fisheries Resources.

During these meetings, discrepancies in the age determination of small pelagic species in the Adriatic Sea sub-region were pointed out. Consequently, the general need for the harmonization, at Adriatic level, of the fish ageing by otolith reading for anchovy and sardine has been identified by Adriatic experts.

[1] The research institutes participating to the research programme AdriaMed-SP were: Fisheries Research Institute (FRI) Durres, Albania; Institute of Oceanography and Fisheries (IOF), Croatia; Marine Fisheries Research Institute – National Research Council (IRPEM) Ancona, Italy; National Institute of Biology (NIB) Ljubljana, Slovenia.

[2] Ancona (Italy), 8–11/05/2001; Ancona (Italy), 13–17/05 and 20-24/05/2002; Split, (Croatia), 9-12/12/02; Ancona (Italy), 24-28/02/03; Ancona (Italy), 26-29/04/2004

During the AdriaMed Working Group on Small Pelagic Fisheries Resources (Split, Croatia, 11–15 October 2010) the need to carry out an otolith reading calibration exercise, especially on the sardine, was brought forward as a priority issue to be addressed in the short term. The proposal was presented and agreed on by the Twelfth Meeting of the AdriaMed Coordination Committee (Ljubljana, Slovenia, 1st - 2nd March 2011) which included the organisation of a meeting to specifically identify and address the age determination related problems in the Project work plan for 2011.

The AdriaMed Workshop on inter-calibration of sardine age determination through otolith reading (henceforth referred to as WS) was held at Institute of Oceanography and Fisheries in Split (Croatia) on 28 and 29 June 2011.

1.2 Terms of reference

The terms of reference of the AdriaMed Workshop were:

1.. To test and improve the precision of current sardine ageing procedures.
2.. To discuss current otolith preparation methodologies in order to develop a standard for the sub-region.
3..To improve existing ageing methods.
4..To revise the sardine age-reading criteria.
5..To evaluate the agreement among otolith readers and the precision of age determination.
6..To stimulate regional collaboration.

2. Material and methods

2.1 Opening and planning of the workshop

After the presentation of the age reading criteria adopted by different laboratories, readers participated in a plenary discussion. After this joint discussion, readers performed an individual age reading in order to check if readers had understood the agreed criteria. A further discussion on the structure of otolithsthat have raised more difficulties in ageing followed. These otoliths were selected among those which showed the lower average agreement with the modal age across all ages and readers. For this purpose an image analysis system was used consisting of a stereomicroscope equipped with a high resolution video camera linked to a PC running the image analysis software (Image Pro-Plus version 7.0), which was connected to a multimedia projector allowing all participants to watch the otolith images on a large screen. Several otoliths were analyzed by all readers to have consensual age estimation. The month of catch was provided and readers were asked to attribute a degree of reliability to each age estimation. Issues concerning first annulus identification, nature of the edge and decreasing ring width were considered. After the individual readings, the otolith was projected on a screen and readers were asked to explain their own age interpretation and the criteria applied. Subsequently, the rules used for sardine age estimation were discussed and common criteria were agreed.

2.2 Otolith preparation for reading

2.2.1 Equipment required and magnification:

Use high-quality optical binocular microscopes with 40x magnification. Use high-intensity lighting, preferably optic fiber lamps.

2.2.2 Preparation for ageing:

For contrast between translucent and opaque zones, submerge otolith in clear media (such as water or alcohol) in a Petri dish with a black background. In this way, translucent zones will appear dark and opaque zones light. The purpose of setting the liquid in the Petri dish is to reduce glare on the three dimensional surface of the otolith.

2.2.3 Axes to count annuli:

View the whole otolith with the concave (distal) surface facing upward. The best axis to count annuli depends on the species. In most cases, the postero-dorsal, posterior and ventral axes are preferred (Figure 1).

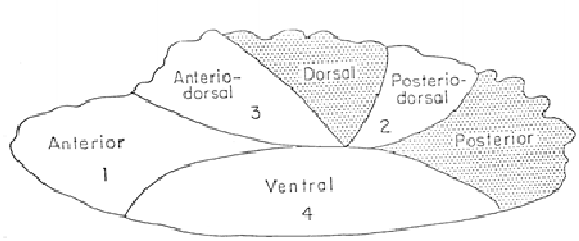

Figure 1 - Schematic presentation of otolith surface.

2.3 Provisional interpretation criteria used for sardine otolith:

- ✓ 1st of January is assigned as the birthdate.
- ✓ The nucleus is hyaline surrounded by the first opaque ring.
- ✓ One opaque and one hyaline ring (annulus) are formed once a year.
- ✓ Annuli width decreases progressively as fish becomes older.
- ✓ Otoliths with no visible hyaline rings are considered to belong to age class 0.

2.4 Validation techniques

Taking advantage of the experience of participants, the methodology used for otolith sampling preparation, reading in different areas of sardine distribution as well as validation technique were discussed during the workshop and are summarized in Table 1.

Table 1- Summary of the otolith sampling, preparation procedures and age reading criteria in different areas.

AREA	SAMPLE	OTOLITH SAMPLING	OTOLITH PREPARATION	AGE DETERMINATION	VALIDATION TECHNIQUES
ITALIAN COAST	Commercial catches; research surveys	In each monthly sample, 5 otolith pairs by length class (0.5 cm).	Stored dried in vials and immersed in alcohol for observation.	Hyaline ring counts and edge type and interpretation with catch date and reference birthdate.	Marginal increment analysis and micro-increment counts (daily rings in o age group)
ALBANIAN COAST	Commercial catches; research surveys	In each monthly sample, 5 otolith pairs by length class (0.5 cm).	Stored dried in vials and immersed in alcohol for observation	Hyaline ring counts and edge type and interpretation with catch date and reference birthdate.	
MONTENEGRIN COAST	Commercial catches; research surveys	In each monthly sample, 5 otolith pairs by length class (0.5 cm).	Stored dried in vials and immersed in alcohol for observation.	Hyaline ring counts and edge type and interpretation with catch date and reference birthdate.	Marginal increment analysis and micro-increment counts (daily rings in o age group)
CROATIAN COAST	Commercial catches	In each monthly sample, 5 otolith pairs by length class (0.5 cm).	Stored dried in vials and immersed in alcohol for observation.	Hyaline ring counts and edge type and interpretation with catch date and reference birthdate.	
SLOVENIAN COAST	Commercial catches	In each monthly sample, 5 otolith pairs by length class (0.5 cm).	Stored dried in vials and immersed in alcohol for observation.	Hyaline ring counts and edge type and interpretation with catch date and reference birthdate.	

2.5 Sardine inter-calibration and comparative age determination

2.5.1 Available otolith samples

Sardine otoliths data set used during the workshop is summarized in Table 2. Overall, 40 otoliths were analysed for age assignment, distributed in 5 sets from different fishing areas (Figure 2).

2.5.2 Guidelines on age reading

During the workshop age readers deeply debated to reach an agreement on ageing methodology. A presentation (see Annex 1) on sardine otolith reading, which was available at the beginning of the meeting, was constantly open for discussion and improvement. At the end of the workshop, the guidelines for sardine otolith reading were discussed and they are presented in section 3.2.

Table 2. Summary of sardine otolith sets used for age reading.

SET-AREA	NUMBER OF OTOLITH	LENGTH RANGE (cm)	YEAR	INSTITUTE
SET-1 (North Adriatic)	8	12.0-17.0	2010	Fisheries Research Institute,Slovenia
SET-2 (Central Adriatic)	8	12.0-17.0	2010	Institute of Oceanography and Fisheries, Split
SET-3 (South Adriatic)	8	12.0-17.0	2010	Institute of marine biology, Kotor,
SET-4 (South Adriatic)	8	12.0-17.0	2010	Aquaculture & Fishery Laboratory, Albania
SET-5 (Central Adriatic)	8	12.0-17.0	2010	ISMAR-CNR of Ancona, Italy

Figure 2 – Sardine otolith sets from different fishing areas in the Adriatic Sea.

3. Results of sardine otolith inter-calibration

3.1 Data analysis

Age estimates obtained on otolith sets reported in Table 2 were used to evaluate the current precision and bias in the sardine age readings.

All data were analyzed using the Workbook Age Reading comparisons of Eltink (2000) and following the recommendations of the Guidelines and tools for age reading comparisons (Eltink *et al.*, 2000). Tables 3,4,5 and 6 respectively show the number of age readings performed by each reader, the coefficient of variation (CV) of readings, the percentage agreement among readers and modal age, the age composition of all otolith sets.

Table 3 – Number of readings.

NUMBER OF AGE READINGS										
MODAL age	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	TOTAL
Age 0	-	-	1	1	-	1	-	-	-	-
Age 1	10	15	16	16	14	15	15	11	14	126
Age 2	9	15	15	15	14	15	15	15	14	127
Age 3	1	2	2	2	2	2	2	2	2	17
Age 4	-	1	1	1	1	1	1	1	1	-
Total	**20**	**33**	**35**	**35**	**31**	**34**	**33**	**29**	**31**	**281**

Table 4 – Coefficient of variation.

COEFFICIENT OF VARIATION (CV)										
MODAL age	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	All Readers
Age 0	-	-	-	-	-	-	-	-	-	-
Age 1	35%	28%	0%	0%	29%	0%	28%	33%	29%	**9%**
Age 2	18%	28%	12%	24%	0%	28%	22%	31%	0%	**12%**
Age 3	-	0%	0%	0%	0%	0%	0%	0%	0%	-
Age 4	-	-	-	-	-	-	-	-	-	-
Weighted mean	**25,5%**	**25,1%**	**5,4%**	**10,4%**	**13,0%**	**12,2%**	**22,6%**	**28,7%**	**13,0%**	**9,4%**
RANKING	**8**	**7**	**1**	**2**	**4**	**3**	**6**	**9**	**4**	

Table 5- Percentage agreement.

PERCENTAGE AGREEMENT										
MODAL age	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	ALL
Age 0	-	-	100%	100%	-	100%	-	-	-	-
Age 1	90%	93%	100%	100%	93%	100%	93%	91%	93%	**95%**
Age 2	89%	93%	93%	73%	100%	93%	80%	87%	100%	**90%**
Age 3	100%	100%	100%	100%	100%	100%	100%	100%	100%	**100%**
Age 4	-	100%	100%	100%	100%	100%	100%	100%	100%	-
Weighted mean	**90,0%**	**93,9%**	**97,1%**	**88,6%**	**96,8%**	**97,1%**	**87,9%**	**89,7%**	**96,8%**	**93,2%**
RANKING	**6**	**5**	**1**	**8**	**3**	**2**	**9**	**7**	**3**	

Table 6 – Age composition.

AGE COMPOSITION										
	Reader 1	Reader 2	Reader 3	Reader 4	Reader 5	Reader 6	Reader 7	Reader 8	Reader 9	TOTAL
Age 0	1	2	1	1	1	2	1	2	1	**12**
Age 1	10	14	16	17	13	15	15	11	13	**124**
Age 2	8	14	14	11	14	14	12	13	14	**114**
Age 3	1	2	3	5	2	2	4	2	2	**23**
Age 4	-	1	1	1	1	1	1	1	1	**8**
Total	**20**	**33**	**35**	**35**	**31**	**34**	**33**	**29**	**31**	**281**

Table 7 shows the percentage agreement among readers and the bias respect to the modal age. The agreement among readers was always over 85%.

Table 7 - Agreement, Inter-reader bias test and reading against Modal Age bias test.

Inter-reader bias test and reader against MODAL age bias test									
	Reader 1	**Reader 2**	**Reader 3**	**Reader 4**	**Reader 5**	**Reader 6**	**Reader 7**	**Reader 8**	**Reader 9**
Reader 1	90,0	85,0	89,5	78,9	83,3	90,0	73,7	100,0	83,3
Reader 2	-	93,9	90,6	81,3	96,8	97,0	87,5	89,3	96,8
Reader 3	-	-	97,1	91,4	93,5	93,9	84,8	89,3	93,5
Reader 4	-	-	-	88,6	87,1	84,8	93,9	78,6	87,1
Reader 5	-	-	-	-	96,8	93,5	93,5	84,6	100,0
Reader 6	-	-	-	-	-	97,1	84,4	92,9	93,5
Reader 7	-	-	-	-	-	-	87,9	75,0	93,5
Reader 8	-	-	-	-	-	-	-	89,7	84,6
Reader 9	-	-	-	-	-	-	-	-	96,8
MODAL age	-	-	-	-	-	-	-	-	-
-	= no sign of bias (p>0.05)								
*****	= possibility of bias (0.01<p<0.05)								
******	= certainty of bias (p<0.01)								
	percentage of reading agreement between each reader and the MODAL ageExperienced								

The coefficient of variation (CV%), the percentage agreement and the standard deviation (STDEV) plotted against MODAL age are shown in Figure 3.
Looking at Figure 3, the percentage agreement in age groups 1, 2 and 3 was between 90 and 100%, with a low coefficient of variation and standard deviation, indicating a good consistency of ageing criteria adopted by readers and the reliability of readings. The age groups 0 and 4 were not taken into account because of the low number of samples.

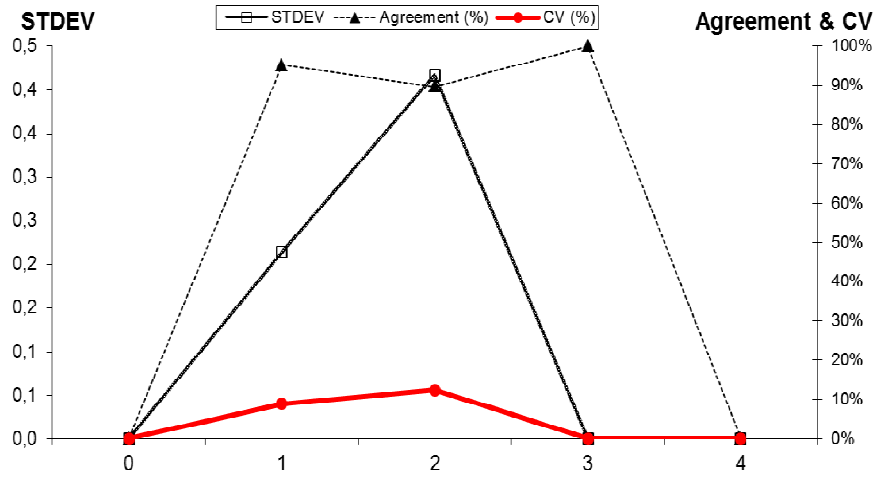

Figure 3 - The coefficient of variation (CV%), percentage agreement and standard deviation (STDEV) vs modal age.

In the age bias plots (Figure 4), the mean age (+/- 2 stdev) is plotted against the modal age for each age reader and all readers combined. The estimated mean age corresponds to the modal

age if it is placed on the solid line (as in the case of modal age 3). The relative bias is the age difference between estimated mean age and modal age.

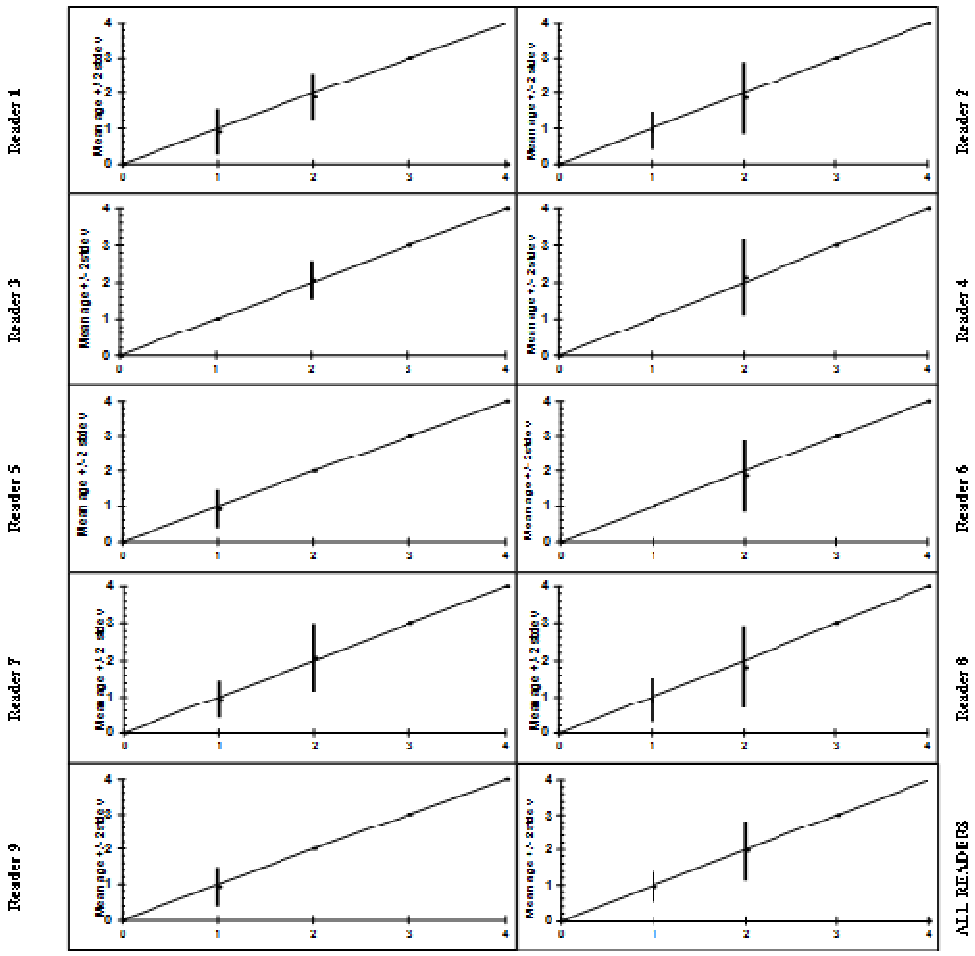

Figure 4 - Sardine otolith inter-calibration of each age reader and all readers combined.

Summarizing results, standard deviation within the modal age 1 was similar in almost all readers, except for 3,4,6 readers who read a few samples in this age group. Standard deviation increased in the modal age 2, except for readers 1 and 2.
Considering the bias (i.e. the systematic error of underestimation or overestimation of the modal age), generally readers 1, 2, 6 and 8 tended to underestimate the age, whereas readers 3, 4 and 7 slightly overestimated the modal age 2.

3.2 Guidelines on age reading after inter-calibration analysis

Another important result obtained during the workshop was to provide common ageing criteria and an age reading protocol, as well as a common database to be used in the countries involved in the workshop.

3.2.1 Ageing criteria

Age determination procedure based on the examination of the sardine otoliths is obtained according to the aforementioned growth pattern and the date of capture, so that the following criteria are met:

1) An annulus consists of one opaque ring and one hyaline ring. Age equals the number of true complete hyaline rings previously defined. One opaque and one hyaline ring are formed once a year.

2) The edge of the otolith should be considered hyaline or opaque if this structure is continuous all around the otolith margin. Anyway, in most cases the edge is discontinuous and it disappears by changing the focus, depending on the sampling season and different otolith areas. Hence, to determine the edge type, it was suggested to focus attention mainly on the post-rostrum area.

Generally, a true hyaline ring should exhibit the following characteristics:

a) be continuous all around the otolith;

b) remain clearly visible changing the focus;

c) the relative distances between adjacent rings are proportional to the expected growth pattern in the otoliths from each area.

3.2.2 Age reading protocol

In order to standardize the sardine age assignments and to improve the age estimates, this workshop adopted the following protocol:

- ✓ 1st of January adopted as the birthdate (Williams and Bedford, 1974). The hyaline rings are generally laid down in winter. Hence, if the fish is caught in the first semester (January-June) with a hyaline otolith edge, the age assigned will be equal to the number of the hyaline rings observed plus 1 (2+h=3 age); if the fish is caught in the second semester (i.e. July-December) with a hyaline otolith edge, the age assigned will be equal to the number of the hyaline rings (2+h=2 age).
- ✓ The observation of the whole otolith has to be made under reflected light against a black background using dissection microscopes with 20-25X magnification.
- ✓ Magnification should be increased near the otolith edge to improve the discrimination of narrow hyaline rings in older individuals.
- ✓ The nucleus is hyaline surrounded by the first opaque ring.
- ✓ Only the hyaline rings are counted.
- ✓ Annuli width decreases progressively as the fish becomes older; width decreases mainly in the first two years of life.
- ✓ Otoliths with no visible hyaline rings are considered to belong to age class 0.
- ✓ For each otolith, the number of true hyaline rings (excluding the edge), edge type, age assigned, as well as false rings (checks) must be recorded.
- ✓ If a faint ring occurs at a distance where a true ring should be expected (based on the diameter of the 1st annulus) it could also be considered as a true ring for age

assignment. Generally the first hyaline ring is laid down approximately between 1-1.5 mm from the primordium and the distance between the first and the second annulus should be approximately 0.15-0.20 mm (FAO Fisheries Report No. 685 ISSN 0429-9337).

- ✓ Since in older specimens growth often slows down to such an extent that hyaline rings are very close each other, they are counted even if they are not continuous all around the otoliths;
- ✓ When hyaline rings are very close to each other forming a cluster (i.e. two or more very close rings), generally appearing in the antirostrum, we consider them as a single ring.
- ✓ Code 99 is used in the case of illegible otolith (broken, poor condition, crystallized).

3.2.3 Development of a future database

The common database with biological data of fish should include the following data:

a. one code which comprises 3 digits for species-SAR, 2 digits for port of catch and 2 digit for sampling year,
b. date of catch,
c. otolith number,
d. total length (cm),
e. total weight (g),
f. age reading (true hyaline rings determined),
g. edge type (hyaline -h or opaque- o),
h. age class group (on the bases of semester of catch and birthdate),
i. distance between nucleus and first hyaline ring (if available),
k. otolith picture (*.tiff, if available)

4. Discussion

The results of the workshop have certainly not solved all the problems and uncertainties involved in ageing sardine specimens. However, it allowed researchers to share their own experiences and to discuss their main concerns in estimating the age of the species.

The majority of readers involved in the workshop showed improved convergences in the sardine otolith interpretation for age determination. The participants agreed that for the correct identification of the first ring its measurement can be very useful. Less experienced readers generally showed a tendency to consider a false ring that appears before the first true annulus as the first annulus. During the workshop it was established that, to solve this problem on the first true hyaline ring, the FAO report on age determination of sardine published in 2001 (FAO Fisheries Report No. 685 ISSN 0429-9337) should be considered as a reference.

The plenary discussion with otolith images undertaken before the otolith readings was very useful, allowing readers to better understand the correct age reading interpretation.

5. Conclusion, recommendations and development of future work

In conclusion, it is worthwhile to emphasize that the AdriaMed workshop on Inter-calibration of Sardine Otolith Reading in the Adriatic Sea should mark a starting point for stimulating regional collaboration, in order to routinely assess the agreement among otolith readers from different laboratories and to further improve the precision and accuracy of age determination for the sardine.
In terms of technical aspects, according to the participants, one of the objective to be pursued in the short term could be the definition of a single von Bertalanffy growth curve for the sardine population in the Adriatic Sea.

The following are the proposed future developments and recommendations:

1) To organise exchange of sardine otoliths on a regular basis.
2) To create a sardine otolith reference collection for the Adriatic Sea. The great importance of the existence of otolith reference collections that allow age readers to improve their readings was also pointed out during the workshop. This may also be extended to the collection of pictures of otoliths. In this view, special care should be taken in the preparation and selection of images to be processed. Images with unclear otolith areas lead to huge differences in age estimation.
3) To analyze the temporal evolution of the otolith edge, in order to validate the annual deposition of annuli. Similarly, this analysis might also allow to gain information about possible causes involved in the formation of false rings (starvation or changes in feeding regime, stress, spawning check, etc..).
4) To establish a medium distance between the primordium and the first ring observed to facilitate readers in establishing if this first ring observed is a check or an annual ring.
5) To promote the identification of the first annulus through the micro-increment daily rings analysis and measurements.
6) The use of cross-readings among readers from different institutes could be necessary to avoid (or to point out) differences in the reading criteria and to correct them.
7) To carry out this type of activity better it would be desirable to organize *ad hoc* workshops annually at least.

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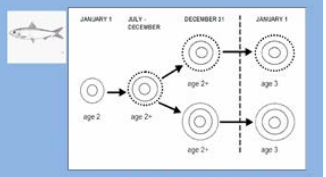
Annex 2 - Contribution to the workshop

Provisional interpretation criteria used:

- ✓ The birth date is assigned to be 1st January.
- ✓ The nucleus is hyaline surrounded by the first opaque ring.
- ✓ One opaque and one hyaline rings (annulus) are formed once a year.
- ✓ Annuli width decrease progressively as fish becomes older.
- ✓ Otoliths with no visible hyaline rings are considered to belong to age class 0.

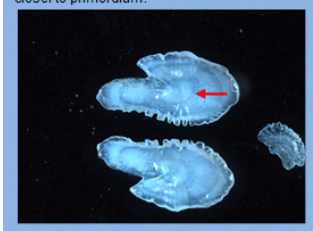
Deposition of hyaline edge and determination of age class



December 3 hyaline rings = age class 2
January 3 hyaline rings= age class 3

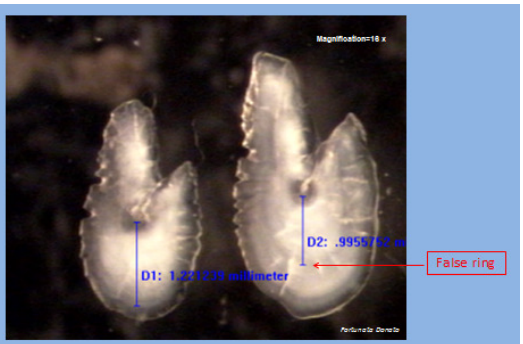
1. If sardine is caught between July and December, with hyaline otolith edge, the age assigned will be equal to the number of the hyaline rings minus 1. (2+h=2 age).
2. If sardine is caught between January and June with a hyaline otolith edge, the age assigned will be equal to the number hyaline rings observed. (2+h=3 age)

Once readings and criteria for age determination are determined, it is important to recognize **the first true hyaline ring**. Often, a very clear and regular hyaline ring can be observed close to the nucleus, it has to be considered false because too closer to primordium.



To solve this problem on the first true hyaline ring, we can refer to the FAO report on age determination of sardine published in 2001, (**FAO Fisheries Report No. 685 ISSN 0429-9337**), where some authors assumed that:

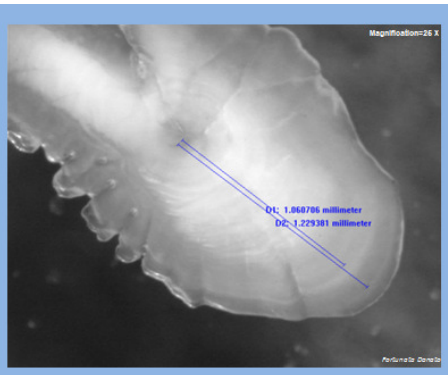
1. Generally the first hyaline ring should have between approximately 1 mm and 1.5 mm.
2. In some cases, it was observed that sardines of 6-11 cm in total length, exhibit 2-5 hyaline false rings, as they were aged 60-220 days by means of micro increment counts.
3. Ring width decreases mainly after the first 2 years of life.



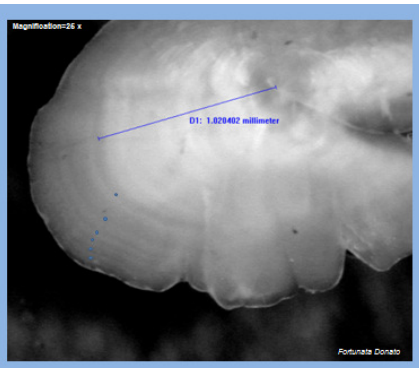
sardine caught in March, TL(cm)=11.5 – Age reading 0h - Age class 1

sardine caught in March, TL(cm)=14.5, Age reading 1h, Age class 2

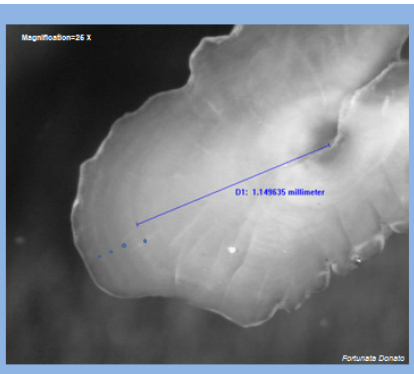
sardine caught in March, TL(cm)=16.0, Age reading 1h, Age class 2



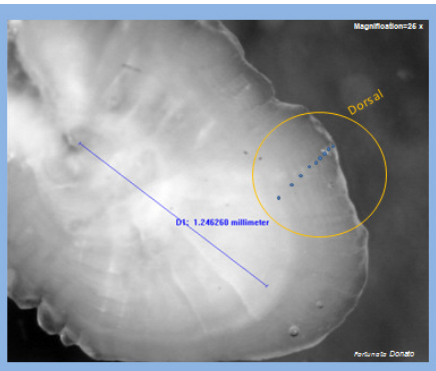
sardine caught in March, TL(cm)=16.5, Age reading 2h, Age class 3



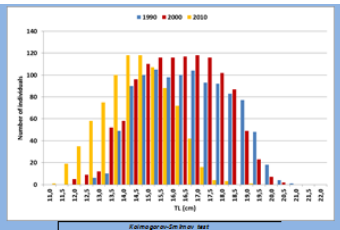
sardine caught in January, TL(cm)=20.5, Age reading 6h, Age class 7



sardine caught in March, TL(cm)=18.0, Age reading 4h, Age class 5



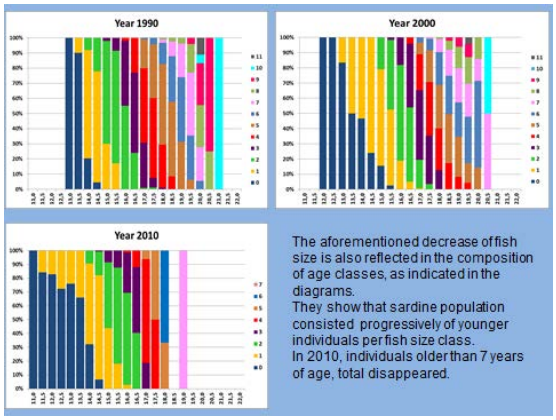
sardine caught in January, TL(cm)=19.5, Age reading 9h, Age class 10



Kolmogorov-Smirnov test

Year	D	0.025	0.01	# *=0.05	#**=0.01
1990 vs 2000	0.07	0.05	0.06		**
2000 vs 2010	0.45	0.06	0.07		**
1990 vs 2010	0.50	0.06	0.07		**

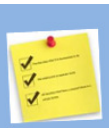
Length frequency distributions of 3 sampling years are summarized in the figure above. It should be noted that among 1990, 2000 and 2010, the fish size distributions are significantly different each other.
In 1990 the size distributions was between 13 and 21 cm, whereas in to 2000 the size distribution was slightly narrower, between 12 and 20.5 cm.
In 2010 the population consisted of considerably smaller individuals than previous years , ranging between 11 to 19 cm.
In the table is shown the statistical significance of these differences.



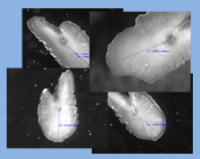
The aforementioned decrease of fish size is also reflected in the composition of age classes, as indicated in the diagrams.
They show that sardine population consisted progressively of younger individuals per fish size class.
In 2010, individuals older than 7 years of age, total disappeared.

Future work

- To follow the criteria for sardine age determination established in this workshop.

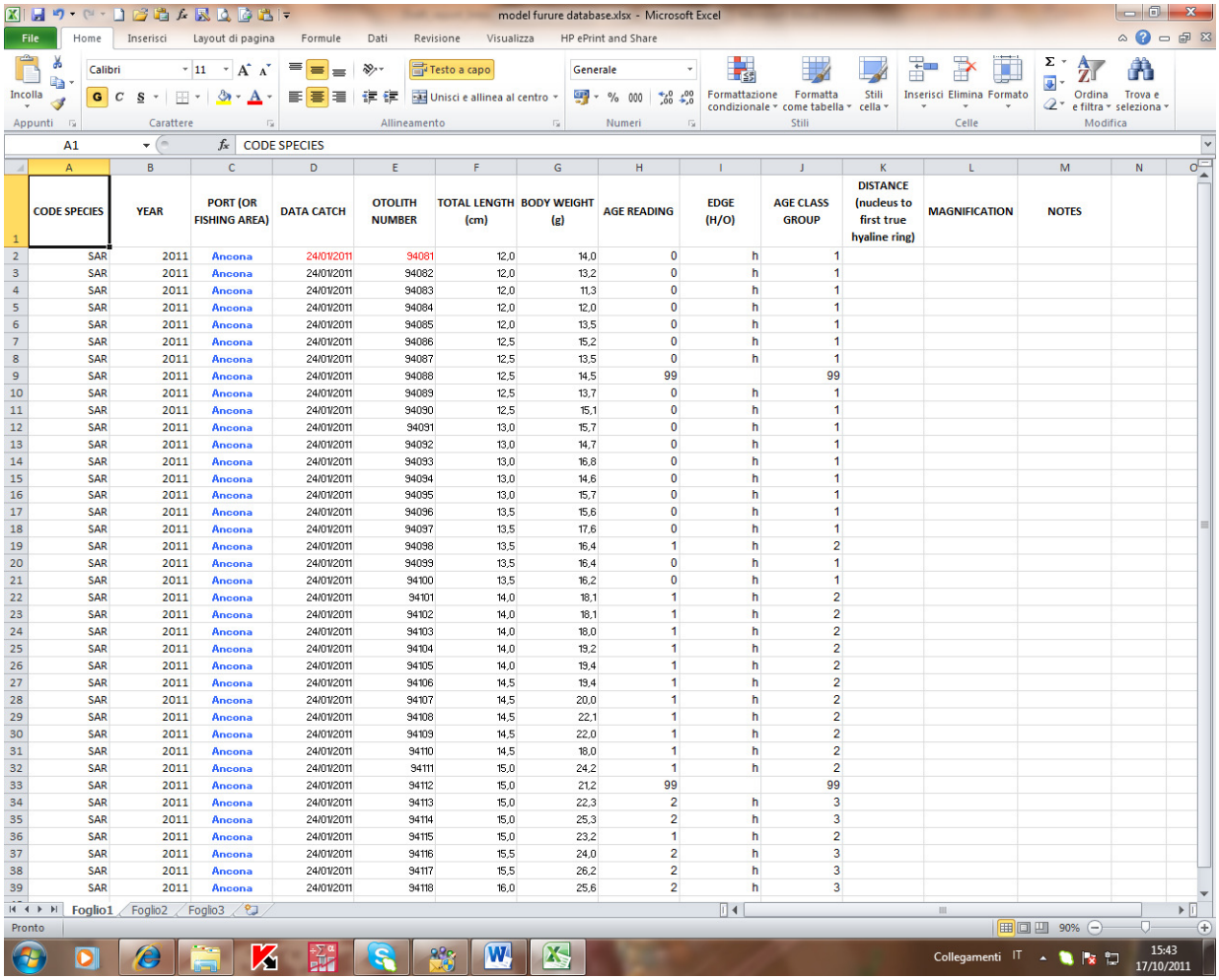


- To create a photographic atlas of sardine otoliths by age class, including images of otoliths collected from all countries involved in the AdriaMed project.



- To provide age validation by daily rings analysis

Annex 3- Common model of the future database



model furure database.xlsx - Microsoft Excel

CODE SPECIES	YEAR	PORT (OR FISHING AREA)	DATA CATCH	OTOLITH NUMBER	TOTAL LENGTH (cm)	BODY WEIGHT (g)	AGE READING	EDGE (H/O)	AGE CLASS GROUP	DISTANCE (nucleus to first true hyaline ring)	MAGNIFICATION	NOTES
SAR	2011	Ancona	24/01/2011	94081	12,0	14,0	0	h	1			
SAR	2011	Ancona	24/01/2011	94082	12,0	13,2	0	h	1			
SAR	2011	Ancona	24/01/2011	94083	12,0	11,3	0	h	1			
SAR	2011	Ancona	24/01/2011	94084	12,0	12,0	0	h	1			
SAR	2011	Ancona	24/01/2011	94085	12,0	13,5	0	h	1			
SAR	2011	Ancona	24/01/2011	94086	12,5	15,2	0	h	1			
SAR	2011	Ancona	24/01/2011	94087	12,5	13,5	0	h	1			
SAR	2011	Ancona	24/01/2011	94088	12,5	14,5	99		99			
SAR	2011	Ancona	24/01/2011	94089	12,5	13,7	0	h	1			
SAR	2011	Ancona	24/01/2011	94090	12,5	15,1	0	h	1			
SAR	2011	Ancona	24/01/2011	94091	13,0	15,7	0	h	1			
SAR	2011	Ancona	24/01/2011	94092	13,0	14,7	0	h	1			
SAR	2011	Ancona	24/01/2011	94093	13,0	16,8	0	h	1			
SAR	2011	Ancona	24/01/2011	94094	13,0	14,6	0	h	1			
SAR	2011	Ancona	24/01/2011	94095	13,0	15,7	0	h	1			
SAR	2011	Ancona	24/01/2011	94096	13,5	15,6	0	h	1			
SAR	2011	Ancona	24/01/2011	94097	13,5	17,6	0	h	1			
SAR	2011	Ancona	24/01/2011	94098	13,5	16,4	1	h	2			
SAR	2011	Ancona	24/01/2011	94099	13,5	16,4	0	h	1			
SAR	2011	Ancona	24/01/2011	94100	13,5	16,2	0	h	1			
SAR	2011	Ancona	24/01/2011	94101	14,0	18,1	1	h	2			
SAR	2011	Ancona	24/01/2011	94102	14,0	18,1	1	h	2			
SAR	2011	Ancona	24/01/2011	94103	14,0	18,0	1	h	2			
SAR	2011	Ancona	24/01/2011	94104	14,0	19,2	1	h	2			
SAR	2011	Ancona	24/01/2011	94105	14,0	19,4	1	h	2			
SAR	2011	Ancona	24/01/2011	94106	14,5	19,4	1	h	2			
SAR	2011	Ancona	24/01/2011	94107	14,5	20,0	1	h	2			
SAR	2011	Ancona	24/01/2011	94108	14,5	22,1	1	h	2			
SAR	2011	Ancona	24/01/2011	94109	14,5	22,0	1	h	2			
SAR	2011	Ancona	24/01/2011	94110	14,5	18,0	1	h	2			
SAR	2011	Ancona	24/01/2011	94111	15,0	24,2	1	h	2			
SAR	2011	Ancona	24/01/2011	94112	15,0	21,2	99		99			
SAR	2011	Ancona	24/01/2011	94113	15,0	22,3	2	h	3			
SAR	2011	Ancona	24/01/2011	94114	15,0	25,3	2	h	3			
SAR	2011	Ancona	24/01/2011	94115	15,0	23,2	1	h	2			
SAR	2011	Ancona	24/01/2011	94116	15,5	24,0	2	h	3			
SAR	2011	Ancona	24/01/2011	94117	15,5	26,2	2	h	3			
SAR	2011	Ancona	24/01/2011	94118	16,0	25,6	2	h	3			